

Effects of Predation by Invasive Western Mosquitofish (*Gambusia affinis*) on Survival of Eggs, Embryos and Tadpoles of *Pelophylax nigromaculatus* and *Duttaphrynus melanostictus* in South China

Xiaoli FAN, Zhihua LIN*, Xiang LI, Li WEI and Guohua DING

College of Ecology, Lishui University, Lishui 323000, China

Abstract Alien species are one of the most serious threats to the decline and extinction of native amphibian populations. In this study, we examined the predation of invasive Western Mosquitofish *Gambusia affinis* on the eggs, embryos, and tadpoles of *Duttaphrynus melanostictus* and *Pelophylax nigromaculatus* in south China. Our results suggested that the survival of eggs and embryos remaining in the egg capsules of *P. nigromaculatus* and *D. melanostictus* was significantly higher than those removed from the egg capsule at 12-h intervals within 72 h in the presence of *G. affinis*. The survival of *P. nigromaculatus* eggs and embryos without egg capsules was significantly lower than those of *D. melanostictus* without egg capsules. The survival of *P. nigromaculatus* eggs and embryos with egg capsules was significantly higher than those of *D. melanostictus* with egg capsules from 24 h to 72 h except for 12 h. The survival of *D. melanostictus* tadpoles was significantly higher than that of *P. nigromaculatus* tadpoles in the presence of *G. affinis*. The survival of Gosner stage 26 tadpoles of *P. nigromaculatus* was significantly higher than that of Gosner stage 30 tadpoles from 12 h to 60 h, but there were no significant differences at 72 h. In contrast, the survival of Gosner stage 26 tadpoles of *D. melanostictus* was significantly lower than that of Gosner stage 30 tadpoles within 72 h, recording every 12h. The increasing temperature caused a significant increase in predation by *G. affinis* on *P. nigromaculatus* eggs and embryos. The outer jelly capsule surrounding anurans eggs might serve as a mechanical defense against predation by *G. affinis* due to its large diameter, relatively stationary state and unpalatability. The differences in the vulnerability of *P. nigromaculatus* and *D. melanostictus* embryos and tadpoles to *G. affinis* probably due to differences in the unpalatability, black skin and activity. Based on the magnitude of predation by *G. affinis* on the eggs, embryos and tadpoles of these two species and the combined impact of temperature, we might speculate that invasive *G. affinis* and global warming would have more detrimental impacts on population viability of *P. nigromaculatus* than *D. melanostictus* in China.

Keywords amphibian anura, tadpoles, eggs, embryos, predation risk

1. Introduction

Alien species are a potential threat to the survival of amphibian larvae globally. In particular, the introduction of non-native fish often results in the rapid decline and extinction of native amphibian populations (Collins and Storfer, 2003; Hussain and Pandit, 2012). Such non-native predators are more of a threat to native species

because native species are often incapable of recognizing non-native predators (Gomez-Mestre and Díaz-Paniagua, 2011; Polo-Cavia and Gomez-Mestre, 2014; Salo *et al.*, 2007; Smith *et al.*, 2007). Western Mosquitofish, *Gambusia affinis*, one of the most widespread introduced fish, is native to the Atlantic coast of North America (Lowe *et al.*, 2000; Pyke, 2005). Their widespread introduction can be attributed to their purported effectiveness at consuming larval mosquitoes (Pyke, 2008). *G. affinis* was introduced to China for this reason in 1927 and has now become widespread throughout the southern water bodies of the Yangtze River (Li and

* Corresponding author: Prof. Zhihua LIN, from Lishui University, Zhejiang, China, with his research focusing on physiological ecology of amphibians and reptiles.

E-mail: zhlin1015@126.com

Received: 28 June 2015 Accepted: 3 November 2015

Jie, 2002). Previous studies have shown that *G. affinis* readily preys on amphibian eggs and larvae, both within (Grubb, 1972; Baber and Babbitt, 2003; Gunzburger and Travis, 2005; Zeiber *et al.*, 2008; Kerby *et al.*, 2012) and outside of its native range (Komak and Crossland, 2000; David and Craig, 2006; Gregoire and Gunzburger, 2008; Segev *et al.*, 2009; Shulse and Semlitsch, 2014). The introduction of *G. affinis* caused a serious threat to the diversity and population dynamics of amphibians, leading the International Union for Conservation of Nature to list it among the 100 worst invasive species (Lowe *et al.*, 2000). To date, there have been no intensive studies on the degree of damage caused to Chinese anurans by the invasive *G. affinis*.

There is an abundance of vegetation and water bodies at the Lishui University campus (latitude 28°27' N, longitude 119°53' E) situated in Zhejiang, South China. The advantageous environment provides living and breeding habitats for a large number of amphibians. Among them, *Duttaphrynus* (formerly *Bufo*) *melanostictus* and *Pelophylax nigromaculatus* breed in the same permanent ponds (Figure 1A) from March to May every year, so the two species both belong to spring-breeders. However, there are some differences in their clutch structure. The amplexant pairs of *D. melanostictus* spawn long egg strings entwining the vegetation, including

Nymphaea alba, *Hydrilla verticillata*, and *Alternanthera philoxeroides* (Figure 1B). In contrast, the amplexant pairs of *P. nigromaculatus* spawn globular egg masses, and this species lays a single egg mass on vegetative cover (Figure 1C) or a large number of egg masses in the open water (Figure 1D). However, their fertilized eggs are enveloped by transparent jelly capsules. The invasive *G. affinis* also inhabits in the breeding ponds, so the eggs, embryos, and tadpoles of these two anurans may face predation risk.

In this study, we examined the survival of the eggs, embryos, and tadpoles of *D. melanostictus* and *P. nigromaculatus* exposed to the visual cues of the invasive predator *G. affinis*. Our aims were to evaluate (1) whether the jelly capsule of eggs plays a role in protecting from the predators, (2) whether there are differences in *G. affinis* predation on different development stages of tadpoles, and (3) the effect of temperature on predation.

2. Materials and Methods

2.1 Collection of experimental animals Three freshly laid *P. nigromaculatus* egg masses and three *D. melanostictus* egg strings were collected from a permanent pond at Lishui University on March 22, 2014. All of the eggs were carried back to our herpetological laboratory. From each family, about 1200 eggs were directly used in two experiments, the remaining eggs of each species



Figure 1 Breeding water body and egg clutch of *Duttaphrynus melanostictus* and *Pelophylax nigromaculatus*, (A) a permanent pond, (B) amplexing and spawning of *D. melanostictus*, (C) single egg mass of *P. nigromaculatus* in the vegetative cover, (D) multiple egg masses of *P. nigromaculatus* in open water.

were separately incubated in two 700 mm × 500 mm × 400 mm (length × width × height) plastic containers filled with aged tap water to a 200 mm depth until tadpoles reached Gosner stages 26–30 (Gosner, 1960). The development stages of tadpoles were determined using an anatomical microscope (Nikon XTS30). The tadpoles were fed commercial fish food ad libitum every 2 days. The same pond retains water and contains *G. affinis* year-round. We collected approximately 350 similar in size female *G. affinis* with black embryo spots on both ventral sides using nets (mesh size: 2 mm) (Deaton and Cureton, 2011; Pyke, 2005). They were transferred to an outdoor pool (3.0 m × 2.0 m × 1.5 m) to be raised. *G. affinis* were starved for 24 h before the experiment.

Eggs with or without jelly capsules of *P. nigromaculatus* and *D. melanostictus* were randomly selected and transferred into Petri dishes (60 mm diameter, 10 mm depth) labeled with size standards, and photographed from above using a digital camera (Sony DSC-T100), then their egg diameters and jelly capsules diameters were analyzed using ImageJ 1.44p software (to the nearest 0.01 mm) (Fan *et al.*, 2014).

2.2 Experimental design

Predation of *G. affinis* on eggs and embryos of *P. nigromaculatus* and *D. melanostictus* Between March 22 and 25, 2014, ten eggs with a jelly coat or ten eggs without a jelly coat (jelly coats were removed by slowly sucking out fertilized eggs with a capillary tube) of *P. nigromaculatus* and *D. melanostictus* were settled into separate plastic bowls. Plastic bowls with a 150 mm diameter were filled with water depth of approximately 80 mm and 30 replicates each treatment. After these were done, a fish was randomly assigned to each bowl with a 150 mm diameter. We estimated survivorship by counting the number of eggs or embryos remaining in the bowls of all treatments at 12 h intervals for consecutive 72 h. The environmental conditions in the laboratory were 12 L : 12 D photoperiod with an air temperature of $25 \pm 1^\circ\text{C}$ (mean \pm SE).

Predation of *G. affinis* on different stage tadpoles of *P. nigromaculatus* and *D. melanostictus* Between 2–9 April, 2014, we performed a completely randomized 2 × 2 factorial design with 4 replicates of each treatment. The experiment had two tadpole levels (*P. nigromaculatus* and *D. melanostictus*) crossed with two levels of tadpole initial Gosner stage: 26 or 30. Tadpoles were firstly randomly assigned to each treatment, and then a similar sized fish was transferred into every bowl. We recorded the survival of tadpoles in each treatment by the same method above.

Predation of *G. affinis* on eggs and embryos of *P. nigromaculatus* at different temperatures According to data consecutively recorded hourly by HOBO temperature data loggers (USB, U12-006) in outdoor laboratory round-year, the average minimum and maximum air temperature from March to April in 2013 were 15.2 and 25.3°C, respectively, which were used as the basis of our experiment temperature designs. On 22 March, 2014, we firstly placed ten eggs into fifteen plastic bowls, later placed fifteen fish into the same bowls. The environment chambers were set at 15°C, 20°C, and 25°C, and the photoperiod was 12L: 12D. The survival of tadpoles in each treatment was recorded using the same method as above.

All of the eggs, embryos, tadpoles and fish of each treatment were used only once. After the experiments, excess eggs and tadpoles were released back to the site of capture. All of the *G. affinis* were frozen to death, and their body length (the distance from snout to cloaca) and snout width (the distance between left and right jaw angles) were measured by a digital caliper (to the nearest 0.01 mm).

2.3 Statistical analysis The mean survival number of anuran eggs, embryos and tadpoles every 12 hours was calculated for each treatment. All statistical analyses were performed using Statistica 5.0 software. All variables were tested for normality and homogeneity using the Kolmogorov-Smirnov test and F-max test, respectively. Due to the inhomogeneity of variance (all $P < 0.001$), we used the Kruskal-Wallis test to examine the differences in the survivorship of *P. nigromaculatus* and *D. melanostictus* eggs, embryos (with and without egg capsules) and tadpoles (Gosner stage 26 and 30) coexisting with free-roaming *G. affinis* at 12 h intervals within 72 h in the first and second experiments. Data for the survival of *P. nigromaculatus* eggs and embryos in the presence of *G. affinis* at three temperature treatments (15°C, 20°C, and 25°C) were compared with One-way MANOVA and Tukey's *post hoc* test with different times as factor. All results were expressed as a mean \pm SE, with $\alpha = 0.05$ considered as statistically significant.

3. Results

G. affinis used in the experiments had a mean body length of 35.70 mm (SE = 0.49, range = 30.81 to 44.22 mm) and snout width of 2.86 mm (SE = 0.06, range = 3.03 to 4.82 mm). Egg diameter of *P. nigromaculatus* was an average of 1.53 ± 0.03 (1.17–1.67) mm, and jelly capsules diameter was an average of 5.47 ± 0.08 (4.67–5.84) mm.

Egg diameters of *D. melanostictus* was an average of 1.50 ± 0.13 (1.16–1.70) mm.

3.1 Predation of *G. affinis* on eggs and embryos of *P. nigromaculatus* and *D. melanostictus* Kruskal-Wallis test revealed that the survival of *P. nigromaculatus* and *D. melanostictus* eggs and embryos remaining in an egg capsule was significantly higher than those removed from the egg capsule at different times within 12–72 h as shown in Figure 2 (all $P < 0.007$). The survival of *P. nigromaculatus* eggs and embryos without egg capsules was significantly lower than that of *D. melanostictus* without egg capsules in the same time (all $P < 0.007$, Figure 2). The survival of *P. nigromaculatus* eggs and embryos with egg capsules was significantly higher than those of *D. melanostictus* with egg capsules within later 24–72 h (all $P < 0.021$), but there were no significant differences for their survival at early 12 h ($H_{1, N=60} = 2.03$, $P = 0.158$, Figure 2).

3.2 Predation of *G. affinis* on tadpoles of *P. nigromaculatus* and *D. melanostictus* Kruskal-Wallis test indicated that the survival of Gosner stage 26 tadpoles of *P. nigromaculatus* was significantly higher than that of Gosner 30 tadpoles at 12 h intervals within first 12–60 h (all $P < 0.010$), but there was no significant difference during later 60–72 h ($H_{1, N=60} = 1.24$, $P = 0.265$, Figure 3). In contrast, the survival of Gosner stage 26 tadpoles was significantly lower than that of Gosner stage 30 tadpoles of *D. melanostictus* at 12 h intervals within 12–72 h (all $P < 0.040$, Figure 3). The survival of *D. melanostictus* tadpoles was significantly higher than that of *P. nigromaculatus* tadpoles at all different times within 72 h (all $P < 0.001$, Figure 3).

3.3 Predation of *G. affinis* on eggs and embryos of *P. nigromaculatus* at different temperatures One-way MANOVA indicated that temperature had a significant effect on the predation of *G. affinis* on *P. nigromaculatus* eggs and embryos (Wilks' $\lambda = 0.18$, $df = 20, 66$, $P < 0.001$, Figure 4). Tukey's *post hoc* test suggested that there were no significant differences in the survival among 15°C, 20°C, and 25°C treatments at the point of 12 h and 24 h as shown in Figure 4. The survival of *P. nigromaculatus* embryos at 25°C was significantly lower than that at both 15°C and 20°C, while there were no significant differences in the survival between 15°C and 20°C at the point of 36 h and 48 h (Figure 4). The survival of *P. nigromaculatus* embryos at 20°C and 25°C were significantly lower than that at 15°C, however, there were no significant differences in the survival between 20°C and 25°C from 60 h to 120 h (Figure 4).

4. Discussion

Many anuran species enclose their fertilized eggs within transparent capsules which can attenuate environmental thermal variation (Méndez-Narváez *et al.*, 2015), prevent damage from ultraviolet rays (Ovaska *et al.*, 2011), protect the eggs from the harmful effects of water pollutants (Marquis *et al.*, 2006), and serve as a mechanical defense against predation (Grubb, 1972; Zeiber *et al.*, 2008). In our experiments, *G. affinis* consumed rarely the eggs with jelly coats of *P. nigromaculatus* and *D. melanostictus*, and the survival of their embryos was higher than those without jelly coats, so this perhaps confirm the outer jelly capsule of anurans eggs may play an important role in defending against predators to a certain degree. Zeiber *et al.* (2008) found *G. affinis* couldn't consume the eggs of *Rana sylvatica*, *Bufo americanus*, *Ambystoma tigrinum*, and *Pseudacris triseriata triseriata*, and only

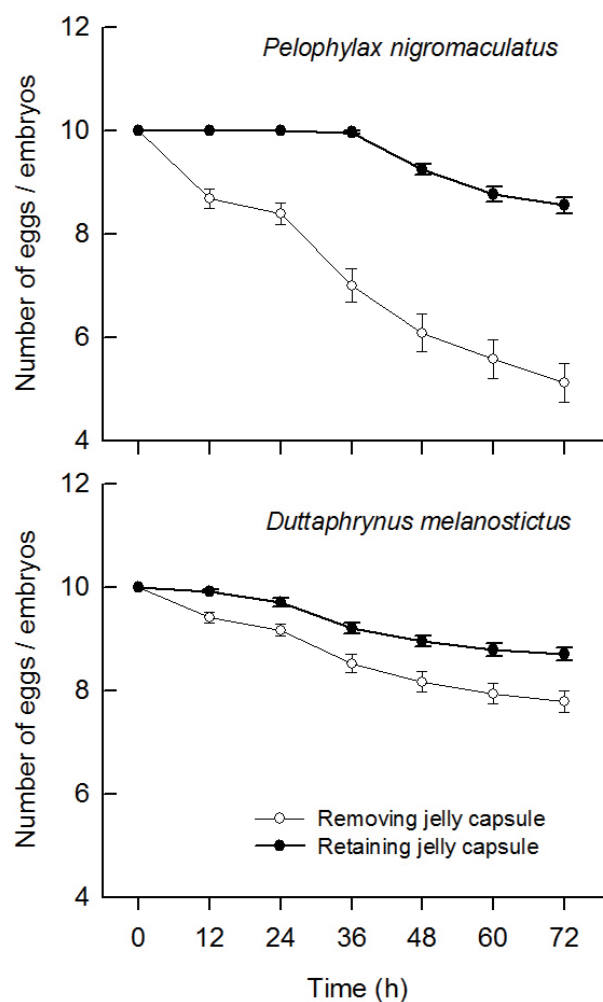


Figure 2 The survival of eggs and embryos of *Pelophylax nigromaculatus* and *Duttaphrynus melanostictus* coexisting with predator *Gambusia affinis* at 12 h intervals within 3 days.

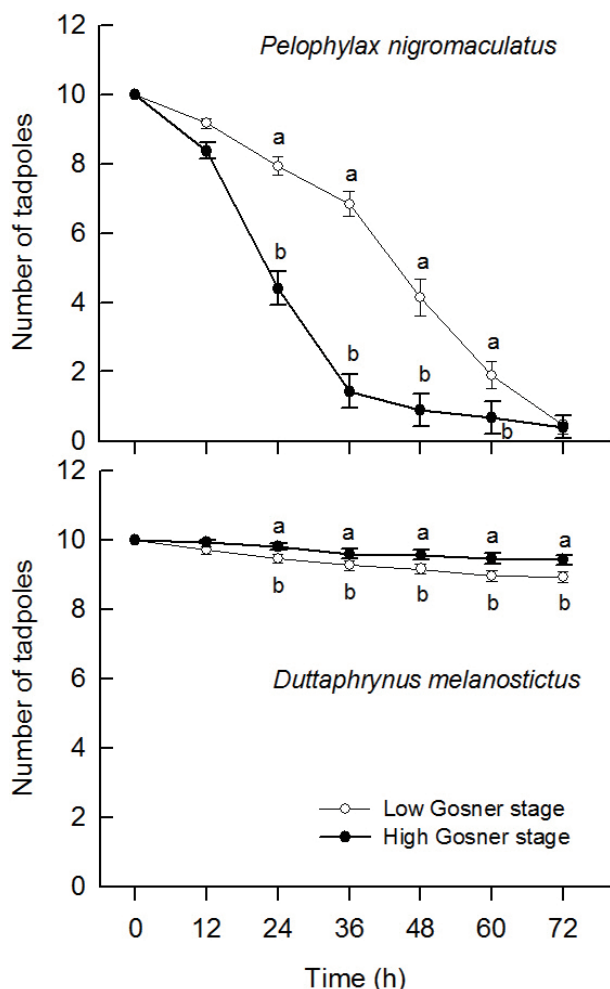


Figure 3 The survival of eggs and embryos of *Pelophylax nigromaculatus* and *Duttaphrynus melanostictus* coexisting with predator *Gambusia affinis* at 12 h intervals within 3 days.

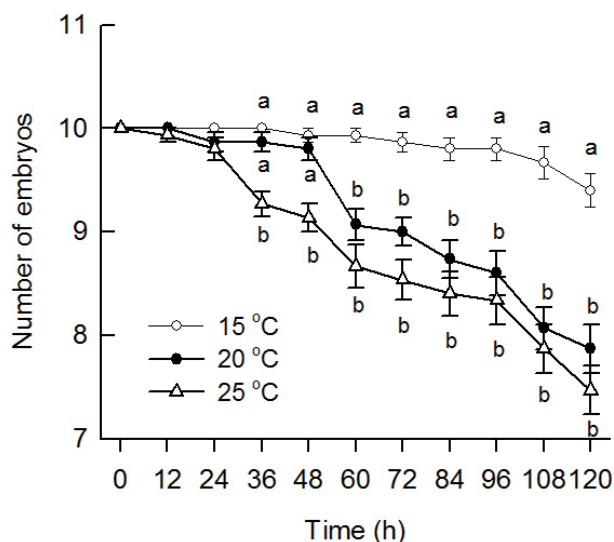


Figure 4 The survival of eggs and embryos of *Pelophylax nigromaculatus* coexisting with predator *Gambusia affinis* at 15°C, 20°C, and 25°C at 12 h intervals within 5 days.

observed a large female *G. affinis* temporarily stuck to the outer egg jelly coating, but was unable to successfully consume the egg. Grubb (1972) have demonstrated that predation was higher for smaller (< 3 mm), unattached anuran eggs and those with soft, loose capsules which facilitated egg extraction and consumption. The eggs of *P. nigromaculatus* with jelly capsule had a diameter larger than 3 mm, and they were often laid in clusters, so the firm jelly capsule most likely deterred western mosquitofish predation. The average snout width of *G. affinis* in our study was much shorter than the eggs diameter of *P. nigromaculatus* with egg capsules, so we believe that the failure of predation on the eggs in part, might be due to the morphological defects of *G. affinis*, as a few studies described it a gape-limited predator (McCoy *et al.*, 2012; Touchon and Wojdak, 2014; Zhao *et al.*, 2014). However, there may be another reason is that the fertilized egg within the jelly coat remain stationary, which might lead to be failure to visually attract to potential predators. When the protective jelly layers of *P. nigromaculatus* and *D. melanostictus* eggs slowly began to dissolve together, and the embryos began to come out of egg capsules since about 36h. At this moment, the predator *G. affinis* had access to prey on the embryos. Thus, we conclude that invasive predator *G. affinis* poses little threat to the eggs of *P. nigromaculatus* and *D. melanostictus*, but only for a short period of time.

In our study, *G. affinis* preyed on a larger number of eggs and embryos without jelly capsules and tadpoles of *P. nigromaculatus* than those of *D. melanostictus*, for instance, *G. affinis* consumed nearly 95% *P. nigromaculatus* tadpoles but only 10% *D. melanostictus* ones within 72 h. Thus *G. affinis*'s predation on anuran eggs and larvae might be species dependent. Several species belonging to the family Bufonidae were known to produce noxious or toxic compounds that deter native predators (such as dragonflies, Laurila *et al.*, 1997; Karraker, 2011) or invasive predators (*G. affinis*, Komak and Crossland, 2000; *Pomacea canaliculata*, Karraker and Dudgeon, 2014; *Procambarus clarkii*, Nunes and Richter-Boix, 2013). These toxins might render the larvae unpalatable to these predators, so unpalatability is often used as a common defensive strategy. Several previous studies have shown that the predatory western mosquitofish preyed selectively on palatable tadpoles, such as *Limnodystes ornatus* and *Pseudacris triseriata triseriata*, avoiding unpalatable tadpoles of *Bufo marinus* and *Bufo americanus* (Komak and Crossland, 2000; Zeiber *et al.*, 2008). Moreover, a tadpole's coloration is related to its anti-predator mechanism. Unpalatable

tadpoles usually present black coloration, which is generally associated to aposematism (Heyer *et al.*, 1975; Crossland and Alford, 1998; Hero *et al.*, 2001). Additionally, unpalatable black tadpoles do not show strong reductions in foraging activity upon perceiving predation risk (D’Heursel and Haddad, 1999). The tadpoles of *D. melanostictus* studied in our experiments also have black skin and are fond of continuous swimming under real predation of *G. affinis* (personal observation). In contrast, tadpoles with brown coloration (just as *P. nigromaculatus*; Fei *et al.*, 2009) usually stay motionless in the presence of a predator and moving from one point to another at high speeds if the predator attacks (Heyer *et al.*, 1975; Azevedo-Ramos *et al.*, 1992). Unpalatability mechanisms are the main defensive trait that makes the coexistence of tadpoles and fish possible (Hero *et al.*, 2001) because fish are considered to be the main predators of tadpoles in permanent water bodies (Heyer *et al.*, 1975). Therefore, *G. affinis* may well pose more threat to *P. nigromaculatus* larvae than to *D. melanostictus* larvae.

An interesting phenomenon happened in our laboratory studies is that predatory *G. affinis* selectively consumed the different sizes of *P. nigromaculatus* and *D. melanostictus* tadpoles: more Gosner stage 26 tadpoles of *P. nigromaculatus* were consumed than Gosner stage 30 ones during the experiment, while it was just the opposite for *D. melanostictus* tadpoles in the same predation pressure. This is not consistent with the results of previous studies, western mosquitofish can consume tadpoles of all sizes up to metamorphosis by biting off their tails and visceral sections until the individual is immobilized and easier to consume (Baber and Babbitt, 2003). The reason should be further studied.

Overall, the higher the temperature was, the more the number of *P. nigromaculatus* embryos preyed upon by *G. affinis* was. It might be the results that the protective jelly capsules began to accelerate dissolution with the temperature increasing, which leading to the embryos in the high temperature treatments exposed to the predator early. As a result, it might be speculated that global warming (Araújo *et al.*, 2006) would exacerbate the extent of the damage caused by *G. affinis* predation on the anuran larvae, which finally leading to the decline in anuran population.

In conclusion, the transparent jelly capsule surrounding eggs of *P. nigromaculatus* and *D. melanostictus* can serve as a mechanical defense against predator due to its large diameter, relatively stationary state and unpalatability. Based on the differences in vulnerability

of the eggs, embryos and tadpoles of *P. nigromaculatus* and *D. melanostictus* to predatory *G. affinis*, we conclude invasive *G. affinis* would pose more threat to the larvae of palatable *P. nigromaculatus* than to unpalatable *D. melanostictus* in China, and global warming would exacerbate the damage of predation by *G. affinis* on *P. nigromaculatus* larvae.

Acknowledgements We thank Prof. Xiang JI (Nanjing Normal University) for providing very useful comments on experimental design and earlier versions of the manuscript. This study was funded by grants from the National Natural Science Foundation of China (31270443, 31500329), the Science Foundation of Zhejiang Provincial Committee of Education (Y201534237) and the Scientific Research Foundation of Ph.D., Lishui University (QD1423).

References

- Araújo M. B., Thuiller W., Pearson R. G. 2006. Climate warming and the decline of amphibians and reptiles in Europe. *J Biogeogr*, 33(10): 1712–1728
- Azevedo-Ramos C., Van Sluys M., Hero J. M., Magnusson W. E. 1992. Influence of tadpole movement on predation by odonata naiads. *J Herpet*, 26: 335–338
- Baber M. J., Babbitt K. J. 2003. The relative impacts of native and introduced predatory fish on a temporary wetland tadpole assemblage. *Oecologia*, 136(2): 289–295
- Collins J. P., Storfer A. 2003. Global amphibian declines: sorting the hypotheses. *Divers Distrib*, 9(2): 89–98
- Crossland M. R., Alford R. A. 1998. Evaluation of the toxicity of eggs, hatchlings and tadpoles of the introduced toad *Bufo marinus* (Anura: Bufonidae) to native Australian aquatic predators. *Austral Ecol*, 23: 129–137
- David L. R., Craig A. S. 2006. Assessment of potential impacts of exotic species on populations of a threatened species, White Sands pupfish, *Cyprinodon tularosa*. *Biol Invasions*, 2006, 8(1): 79–87
- Deaton R., Cureton II J. C. 2011. Female masculinization and reproductive life history in the western mosquitofish (*Gambusia affinis*). *Environ Biol Fish*, 92(4): 551–558
- D’Heursel A., Haddad C. F. B. 1999. Unpalatability of *Hyla semilineata* tadpoles (Anura) to captive and free-ranging vertebrate predators. *Ethol Ecol Evol*, 11: 339–348
- Fan X. L., Lin Z. H., Wei J. 2004. Effects of hydroperiod duration on developmental plasticity in tiger frog (*Hoplobatrachus chinensis*) tadpoles. *Zool Res*, 35(2): 124–131
- Fei L., Hu S. Q., Ye C. Y., Huang Y. Z. 2009. Fauna Sinica: Amphibia, Vol. 3, Anura Ranidae. Beijing: Science Press, 320–1328 (In Chinese)
- Gomez-Mestre I., Díaz-Paniagua C. 2011. Invasive predatory crayfish do not trigger inducible defences in tadpoles Ivan. *P Roy Soc B*, 278(1723): 3364–3370
- Goodsell J. A., Kats L. B. 1999. Effect of introduced mosquitofish

- on pacific treefrogs and the role of alternative prey. *Conserv Biol*, 13(3): 921–924
- Gosner K. L.** 1960. A simplified table for staging anuran embryos and larvae with notes of identification. *Herpetologica*, 16(3): 183–190
- Gregoire D. R., Gunzburger M. S.** 2008. Effects of predatory fish on survival and behavior of larval gopher frogs (*Rana capito*) and southern leopard frogs (*Rana sphenoccephala*). *J Herpet*, 42(1): 97–103
- Grubb J. C.** 1972. Differential predation by *Gambusia affinis* on the eggs of seven species of anuran amphibians. *Am Midl Nat*, 88(1): 102–108
- Gunzburger M. S., Travis J.** 2005. Critical literature review of the evidence for unpalatability of amphibian eggs and larvae. *J Herpet*, 39(4): 547–571
- Hero J. M., Magnusson W. E., Rocha C. F. D., Catterall C. P.** 2001. Antipredator defenses influence the distribution of amphibian prey species in the central Amazon rain forest. *Biotropica*, 33: 131–141
- Heyer W. R., McDiarmid R. W., Weigmann D. L.** 1975. Tadpoles, predation, and pond habitats in the tropics. *Biotropica*, 7: 100–111
- Hussain Q. A., Pandit A. K.** 2012. Global amphibian declines: A review. *Internat J Biodivers Conserv*, 4(10): 348–357
- Karraker N. E., Dudgeon D.** 2014. Invasive apple snails (*Pomacea canaliculata*) are predators of amphibians in South China. *Biol Invas*, 16(9): 1785–1789
- Karraker N. E.** 2011. Are toad tadpoles unpalatable: evidence from the behavior of a predatory dragonfly in South China. *Amphibia-Reptilia*, 32(3): 413–418
- Kerby J. L., Wehrmann A., Sih A.** 2012. Impacts of the insecticide Diazinon on the behavior of predatory fish and amphibian prey. *J Herpet*, 46(2): 171–176
- Komak S., Crossland M. R.** 2000. An assessment of the introduced Mosquitofish (*Gambusia affinis holbrooki*) as a predator of eggs, hatchlings and tadpoles of native and non-native anurans. *Wildl Res*, 27(2): 185–189
- Laurila A., Kujasalo J., Ranta E.** 1997. Different antipredator behaviour in two anuran tadpoles: Effects of predator diet. *Behav Ecol Sociobiol*, 40(5): 329–336
- Li Z. Y., Jie Y.** 2002. Invasive alien species in China. Beijing, China: China Forestry Publishing House, 88 pp (In Chinese)
- Lowe S., Browne M., Boudjelas S.** 2000. 100 of the world's worst invasive alien species — A selection from the global invasive species Database. Invasive Species Specialist Group, World Conservation Union
- Marquis O., Millery A., Guittonneau S., Miaud C.** 2006. Toxicity of PAHs and jelly protection of eggs in the common frog *Rana temporaria*. *Amphibia-Reptilia*, 27(3): 472–475
- McCoy M. W., Touchon J. C., Landberg T., Warkentin K. M., Vonesh J.R.** 2012. Prey responses to predator chemical cues: disentangling the importance of the number and biomass of prey consumed. *PLoS ONE*, 7(10): e47495
- Méndez-Narváez J., Flechas S. V., Amézquita A.** 2015. Foam nests provide context-dependent thermal insulation to embryos of three Leptodactylid frogs. *Physiol Biochem Zool*, 88(3): 246–253
- Nomura I. F., Prado V. H. M., Silva F. R., Borges R. E., Dias N. Y. N., Rossa-Feres D. C.** 2011. Are you experienced? Predator type and predator experience trade-offs in relation to tadpole mortality rates. *J Zool*, 144(2): 144–150
- Nunes A. L., Richter-Boix A.** 2013. Do anuran larvae respond behaviourally to chemical cues from an invasive crayfish predator? A community-wide study. *Oecologia*, 171(1): 115–127
- Ovaska K., Davis T. M., Flamarique I. N.** 2011. Hatching success and larval survival of the frogs *Hyla regilla* and *Rana aurora* under ambient and artificially enhanced solar ultraviolet radiation. *Canad J Zool*, 75(7): 1081–1088
- Polo-Cavia N., Gomez-Mestre I.** 2014. Learned recognition of introduced predators determines survival of tadpole prey. *Funct Ecol*, 28(2): 432–439
- Pyke G. H.** 2005. A review of the biology of *Gambusia affinis* and *G. holbrooki*. *Rev Fish Biol Fisher*, 15: 339–365
- Pyke G. H.** 2008. Plague minnow or mosquitofish? A review of the biology and impacts of introduced *Gambusia* species. *Annu Rev Ecol Evol Syst*, 39(1): 171–191
- Salo P., Korpimäki E., Banks P. B., Nordström M., Dickman C. R.** 2007. Alien predators are more dangerous than native predators to prey populations. *P Roy Soc Lond B*, 274(1615): 1237–1243
- Segev O., Mangel M., Blaustein L.** 2009. Deleterious effects by mosquitofish (*Gambusia affinis*) on the endangered fire salamander (*Salamandra atra*). *Anim Conserv*, 12(1): 29–37
- Shulse C. D., Semlitsch R. D.** 2014. Western mosquitofish (*Gambusia affinis*) bolster the prevalence and severity of tadpole tail injuries in experimental wetlands. *Hydrobiologia*, 723(1): 131–144
- Smith G. R., Boyd A., Dayer C. B., Winter K. E.** 2007. Behavioral responses of American toad and bullfrog tadpoles to the presence of cues from the invasive fish, *Gambusia affinis*. *Biol Invas*, 58(5): 743–748
- Touchon J. C., Wojdak J. M.** 2014. Plastic hatching timing by red-eyed treefrog embryos interacts with larval predator identity and sublethal predation to affect prey morphology but not performance. *PLoS ONE*, 9(6): e100623
- Zeiber R. A., Sutton T. M., Fisher B. E.** 2008. Western mosquitofish predation on native amphibian eggs and larvae. *J Freshw Ecol*, 23(4): 663–671
- Zhao J. Y., Yang Y., Xi X. Q., Zhang C. B., Sun S. C.** 2014. Artificial warming facilitates growth but not survival of plateau frog (*Rana kukunoris*) tadpoles in presence of gape-limited predatory beetles. *PLoS ONE*, 9(6): e98252